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Cation Complexing Fluorescence Probes Containing the Benz[c,d]Indole Fluorophore

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ABSTRACT

A series of new cation complexing fluorescence probes is obtained by covalently linking the fluorophore benz[c,d]indole to the nitrogen atom of various macrocyclic receptors which differ in both the number and position of the heteroatoms, oxygen and nitrogen. The synthesis of these molecules, as well as their absorption and emission properties, are presented. A spectroscopic study of their complexation behavior shows that cation complexation is accompanied by spectral shifts of both the absorption and emission bands and an increase in fluorescence intensity and lifetime, even for heavy and transition metal ions often known as fluorescence quenchers.
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Keywords: benz[c,d]indole, macrocycle, fluorophore, receptor, transition metal ions, complexation.

INTRODUCTION

Fluorometric methods have proven to be useful for the detection of metal ions directly in solution as well as in combination with sensor techniques [1, 2]. These analytical applications require flourescence probes which show a sufficient selectivity for specific metal ions. On the other hand, in combination with chromatographic separation techniques and fluorometric detection

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ion exchange chromatography (IEC), fluorescence probes should be rather unselective in order to detect all the ions separated [3, 4]. The determination of non-fluorescent cations requires the use of flourescence probes which undergo a change in fluorescence behavior, i.e. spectral band position and/or fluorescence intensity and lifetime upon cation complexation. For analytical applications well-suited fluorescence probes are modular fluorophore receptor systems consisting of a fluorophore covalently bound to an ion receptor [5–25]. When the π-electron system of the fluorophore and the receptor are conjugated, these intrinsic fluorescence probes commonly show chelation-induced spectral and intensity changes; whereas, in conjugated probes, where the chromophore and the receptor are electronically separated and the fluorescence of the free probe is usually quenched via fast intramolecular processes such as photo-induced electron transfer, only intensity changes occur [5, 6].

Most of the fluorophore receptor systems described in the literature have been employed for the fluorometric detection of protons, alkali and alkaline-earth metal ions [5, 8–17]. Here, regarding intrinsic probes, receptor complexation frequently leads to considerable shifts in absorption, but only moderate changes in the spectral position of the emission band due to cation repulsion in the excited state, and in some cases to an increase in fluorescence intensity, are observed [8–15]. However, only a comparatively small number of fluorescence probes are known where complexation of the receptor to heavy and transition metal ions results in a fluorescence enhancement [6, 7, 18–23]. In contrast to fluorescent complexes of closed shell diamagnetic cations, fluorescent complexes of heavy metal ions or transition metal ions with unfilled subshells are relatively rare because complex interactions such as intramolecular charge transfer, enhanced spin orbit coupling due to the heavy atom effect, and interactions caused by the paramagnetic nature of transition metal ions often result in strong fluorescence quenching [1]. For instance, binding of some anthrylazamacrocycles and -polyamines to the diamagnetic cations Zn^{2+} and Cd^{2+} is accompanied by a strong fluorescence enhancement (CHEF) by a factor of several hundred, whereas the same molecules show chelation-enhanced fluorescence quenching (CHEQ) with known fluorescence quenchers such as Hg^{2+} and Cu^{2+} [6]. Similar effects have been observed by Fabbrizzi *et al.* and Parker *et al.* [24, 25]. To the best of our knowledge, only a very small number of fluorescence probes recently published show CHEF with heavy and transition metal ions including Cu^{2+} and Co^{2+} [18, 20, 21, 23].

In this paper, a study of the spectroscopic and complexation behavior of the newly designed and synthesized fluorescence probes BI-A15C5 (**3**), BI-$A_2$15C5 (**5**), BisBI-$A_2$15C5 (**7**) and the model compound BI-DMA (**9**) is presented. These fluoroionophores, which consist of the fluorophore

benz[c,d]indole covalently linked to a nitrogen atom of some commercially available macrocyclic mono—and diazareceptors, are some of the very few examples for flourescence probes showing a fluorescence enhancement upon binding to heavy metal ions [6, 7, 18–23]. The optical properties of the uncomplexed fluoroionophores and their complexes with various heavy and transition metal ions are studied by absorption spectroscopy as well as static and time-resolved flourometry. The results are compared to the spectroscopic behaviour of the fluorophore bearing a dimethylamino group substituting for the nitrogen-containing macrocycle. For comparison, the changes of the spectral properties of the fluorescence probes upon addition of alkali and alkaline-earth metal ions are monitored. The heavy and transition metal ions investigated were Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Cu^{2+} and M^{2+}.

EXPERIMENTAL

Reagents

1,4,10-Trioxa-7,13-diazacyclopentadecane, 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane, 1-aza-15-crown-5, 1-aza-18-crown-6 were obtained from Merck. All other chemicals for the synthesis were purchased from Aldrich. For the investigation of the complexation behavior of the fluoroionophore the cations were added as perchlorate salts of highest commercially available grade. The acetonitrile used for the spectroscopic measurements was of spectroscopic grade.

Fluoroionophore synthesis

The chemical structures of the synthesized compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, IR, and elemental analysis and their purity was checked by reversed phase HPLC (HPLC setup from Merck-Hitachi; RP18 column; $CH_3CN + H_2O$:75 + 25 as eluent). The NMR spectra were obtained with a 500 MHz NMR spectrometer Varian $Unity_{plus}$ 500. The IR spectra were measured with a Bruker FTIR-Spectrometer IFS66v. The mass spectra were recorded on a Finnigan MAT 95 spectrometer with an ESI-II/ACPI-source for electrospray ionization and the base peaks $[M + Na]^+$ were determined. The melting points (m.p.) measured with a digital melting point analyser IA 9100 (Kleinfeld GmbH) are uncorrected.

Spectroscopic studies

Absorption spectra were recorded on a Carl Zeiss Specord M500 absorption spectrometer. Static fluorescence measurements were carried out with a

Perkin-Elmer LS-50B fluorometer. For the fluorescence experiments at different excitation and emission wavelengths, only dilute solutions with an optical density below 0.1 at the excitation wavelength were used. For the determination of relative fluorescence quantum yields, the optical densities of the dilute solutions at the excitation wavelengths were determined with a 50 mm absorption curvette. For the chosen fluorescence standard, Coumarin 47 in ethanol, a fluorescence quantum yield of 0.50 was assumed [26].

Time-resolved fluorescence measurements

A unique ps laser impulse fluorometer was used for the determination of fluorescence lifetimes. ps Pulses were generated with an argon ion laser pumped Titan: Sapphire laser with active mode locking and second harmonic generation and a time-correlated single photon counting setup was employed for detection (repetition rate 82 MHz IRF 45 ps; laser system by Spectra Physics and GWU; detection system by AMKO, Hamamatsu, Tennelec, and Ortec). For the determination of the longer fluorescence lifetimes of the complexed fluoroinophores, a pulse picker (Spectra Physics) was employed to reduce the repetition rate to 4 MHz. Convolution and fitting of the fluorescence decay data was performed with the global analysis software by Globals Unlimited.[27]

Complexation studies

Stock solutions of the fluorionophore and the dried perchlorate salts of the various cations (24 h at 120°C in vacuum before use) were prepared in spectroscopic grade acetonitrile. Fluoroinophore concentrations of 1×10^{-1} to 1×10^{-5} M were used for all the experiments. The spectroscopic investigations were carried out by preparing a new sample for each cation concentration in order to avoid dilution. The effect of protonation was investigated employing diluted $HClO_4$ (0.01M).

Preparation of fluorescence probes—general procedure

A mixture of 2-methylaminobenz[c,d]indolium iodide **1** and the corresponding receptor containing a free NH-group **2a–2d** was dissolved in 2–10 ml of DMS or DMAA and heated at 110°C (in the cases of compounds **3**, **4**, **7**, and **8** in the presence of triethylamine). The reaction mixture was poured into 50–150 ml 6% aqueous ammonia solution and the product was extracted with chloroform (four times with 50 ml portions). The combined organic phase was washed with water, dried over anhydrous magnesium sulfate, concentrated under reduced pressure and purified by column chromatoaphy on

alumina using benzene as eluent for **3** and **4**, methanol for **5** and **6**, and chloroform for **7**, **8**, and **9**. The eluent was evaporated, a small amount of toluene or ethyl acetate was added to the residue and the solution was filtered in order to remove aluminum oxide. The product was recrystallized.

13-(2′-Benz[c,d]indolyl)-1,4,7,10-tetraoxa-13-azacyclopentadecane (3) was obtained from 1 mmol of **1** and 1.2 mmol of 1-aza-15-crown-5 using 0.3 ml triethylamine in 2 ml DMS. The pale yellow oil obtained after chromatography was recrystallized twice from a 1:3 mixture of benzene and hexane. The yield was 36% m.p. 112–114°C. Found: C, 68·18; H, 7·20; N, 7·53. Calc. for $C_{21}H_{26}N_2O_4$: C, 68·08; H, 7·08; N, 7·56. MS: the base peak [M + Na] was observed at 392·9 (calculated for $C_{21}H_{26}N_2O_4Na$:393·439) the base peak [M + H] was observed at 371·0 (calculated for $C_{21}H_{27}N_2O_4$:371·457). ^{1}H-NMR ($CDCl_3$) δ (ppm) 3·6–4·1 (-CH_2-CH_2-, m, 20H); 7·3–8·0 (Ar-H, m, 6H). ArH: 7·38–7·43 (CH-3,8, m, 2H); 7·25–7·28 (CH-7, dxd, J = 1·66 Hz; J = 5·95 Hz, 1H); 7·55–7·61 (CH-4, t, J = 7·7 Hz, 1H); 7·88–7·90 (CH-5,6, d, J = 7·85 Hz, 2H). IR (KBr) cm^{-1}: 3100–3000 (*v* CH arom.); 3000–2800 (*v* CH); 1550 (vibrations of heteroatom.ring); 1450 (δ CH crown); 1351 (*v* C-N crown); 1115 (*v* C-O-C crown); 824 and 780 (γ CH arom.).

16-(2′-Benz[c,d]indolyl)-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (4) was obtained from 2 mmol of **1** and 2.3 mmol of 1-aza-18-crown-6 using 0.4 ml triethylamine in 4 ml DMS. After column chromatography, the residue was recrystallized from a 3:1 mixture of benzene and hexane, the yield was 27% m.p. 72–73°C. Found: C, 66.70; H, 7.32; N, 6.84. Calc. for $C_{23}H_{30}N_2O_5$: C, 66.64; H 7.29; N, 6.76. MS: the base peak [M + Na] was observed at 437.0 (calculated for $C_{23}H_{30}N_2O_5Na$: 393.492) the base peak [M + H] was observed at 415.0 (calculated for $C_{23}H_{31}N_2O_5$:415.223). ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.6–4.1 (-CH_2-CH_2-, m, 20H); 7.3–8.0 (Ar-H, m, 6H). IR (KBr) cm^{-1}: 3100–3000 (*v* CH arom.); 3000–2750 (*v* CH); 1556 (vibrations of heteroatom.ring); 1446 (δ CH crown); 1348 (*v* C-N crown); 1119 (u C-O-C crown); 822 and 776 (γ CH arom.).

7-(2′-Benz[c,d]indolyl)-1,4,10-trioxa-7,13-diazacyclopentadecane (5) was prepared from 1 mmol of **1** and 2 mmol of 1,4,10-trioxa-7, 13-diazacyclopentadecane in 2 ml of DMAA. After column chromatography the residue was recrystalized twice from cyclohexane. The yield was 32% m.p. 140,5–142°C. Found: C, 68.36; H, 7.49; N, 11.30 Calc. for $C_{21}H_{27}N_3O_3$: C, 68.24; H 7.38; N, 11.37. MS: the base peak [M + Na] was observed at 392.19528 (calculated for $C_{21}H_{27}N_3O_3Na$: 392.19501). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.176 (NH, s, 1H); 2.80–2.83 [-$(CH_2)_2$ NH, t, J = 4.6 Hz, 4H]; 3.60–4.14 (-CH_2-CH_2-, m, 16H); 7.30–7.33 (CH-7, dxd, J = 5.9 Hz, J = 1.8 Hz, 1H); 7.32–7.48 (CH-6,8, m, 2H); 7.60–7.65 (CH-4, t, J = 7.5 Hz, 1H); 7.88–7.90 (CH-5, d, J = 6.5 Hz, 1H); 7.93–7.95 (CH-3, d, J = 8.1 Hz, 1H). ^{13}C-NMR ($CDCl_3$) δ (ppm): 48.712; 52.663, 53.503 (-CH_2-N <); 68.964; 70.194; 70.371;

71.239 (-CH_2-O-); 114.244; 119.622; 125.265; 127.447; 129.437; 129.641; 130.100; 130.956; 152.297; 165.717 (arom. C).

7-(2′-Benz[c,d]indolyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (6) was prepared from 3 mmol of **1** and 6 mmol 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane in 6 ml of DMAA. The purification procedure was the same as for **5**; the yield was 45% m.p. 76–78°C. Found: C, 66.65; H, 7.38; N, 10.22. Calc. for $C_{23}H_{31}N_3O_4$: C, 66.81; H, 7.56; N 10.16. MS: the base peak [M + Na] was observed at 436.3 (calculated for $C_{23}H_{31}N_3O_4Na$: 436.22123). ^{1}H-NMR ($CDCl_3$) δ (pm): 2.041 (NH, s, 1H) 2.73–2.77 [-(CH_2)-NH, t, J = 4.7 Hz, 4H]; 3.52–4.15 (-CH_2-CH_2-, m, 20H); 7.21–7.23 (CH-7, dxd, J = 6.2 Hz, J = 1.4 Hz, 1H); 7.35–7.43 (CH-6,8, m, 2H); 7.52–7.57 (CH-4, t, J = 7.6 Hz, 1H); 7.81–7.83 (CH-5, d, J = 7.1 Hz, 1H); 7.85–7.88 (CH-3, d, J = 8.1 Hz, 1H). ^{13}C-NMR ($CDCl_3$) δ (ppm): 49.330; 50.962 (-CH_2-N<); 70.237; 70.446 (-CH_2-O-); 113.913; 114.090; 119.363; 125.191; 127.349; 129.532; 129.668; 130.231; 131.047; 152.273; 165.668 (arom. C).

7,13-Bis(2′-benz[c,d]indolyl)-1,4,10-trioxa-7,13-diazacyclopentadecane (7) was obtained from 1 mmol of **1**, 0.5 mmol of 1,4,10-trioxa-7,13-diazacyclopentadecane and 0.15 ml of triethylamine in 3 ml of DMAA. The product was recrystallized three times from i-propanol; the yield was 40% m. p. 198–199°C. Found: C, 73.60; H, 6.25; N, 10.44. Calc. for $C_{32}H_{32}N_4O_3$: C, 73.82; H, 6.20; N, 10.76. MS: the base peak [M + Na] was observed at 542.9 (calculated for $C_{32}H_{32}N_4O_3Na$: 543.6); the base peak [M + H] was observed at 521.2551 (calculated for $C_{32}H_{33}N_4O_3$: 521.641). IR (KBr), cm^{-1}: 3100–3000 (ν CH-arom); 3000–2800 (ν CH_2); 1622 (ν C=N); 1540 (vibrations of heteroarom.ring); 1442 (δ CH, crown); 1341(ν C-N, crown); 1109, 1101, 1063 (ν C-O-C, crown); 821, 771 (γ CH-arom). ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.61 (-O-CH_2-CH_2-O-, s, 4H); 3.82–4.09 (>N-CH_2-CH_2-O-, m, 16H); 7.21–7.24 (CH-7,7′, dxd, J = 5.5 Hz; J = 2.1 Hz, 2H); 7.27–7.43 (CH-6,6′,8,8′, m, 4H); 7.49–7.54 (CH-4,4′, t, J = 7.5 Hz, 2H); 7.84–7.87 (CH-3,3′,5,5′, d, J = 8.1 Hz, 4H) ^{13}C-NMR ($CDCl_3$) δ (ppm): 51.8048; 52.4304 (-CH_2-N<); 68.8051; 68.8961; 70.6262; 70.8386 (-CH_2-O-); 114.159; 119.668; 125.593; 127.350; 129.400; 129.787; 130.085; 130.950; 152.068; 166.021 (arom.C).

7,16-Bis(′2-benz[c,d]indolyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (8) was prepared from 4.4 mmol of **1**, 2 mmol 1,4,10,13-tetraoxa-7,16-diazacyclodecane and 0.7 ml triethylamine in 10 ml DMAA. After column chromatography the residue was recrystallized from benzene yielding orange crystals. The yield was 42% m.p. 171–172°C. Found: C, 72.22; H, 6.45; N, 9.94. Calc. for $C_{34}H_{36}N_4O_4$: C, 72.31; H, 6.42; N, 9.92. MS: the base peak [M + Na] was observed at 586.9 (calculated for $C_{34}H_{36}N_4O_4Na$: 587.676), the base peak [M + H] was observed at 564.9 (calculated for $C_{34}H_{37}N_4O_4$: 565.694). ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.65 (-O-CH_2-CH_2-O-, s, 8H); 3.87–4.11 (>N-CH_2-CH_2-O-, m, 16H); 7.22–7.24 (CH-7,7′, dxd, J = 6.2 Hz,

J = 1.3 Hz, 2H); 7.29–7.43 (CH-6,6′,8,8′, m, 4H); 7.50–7.55 (CH-4,4′, t, J = 7.6 Hz, 2H); 7.77–7.94 (CH-5,5′, d, J = 7.1 Hz, 2H); 7.84–7.87 (CH-3,3′, d, J = 8 Hz, 2H). ^{13}C-NMR ($CDCl_3$) δ (ppm): 51.637 (-CH_2-N<); 69.000–70.913 (-CH_2-O-); 114.098; 119.532; 125.227; 127.378; 128.302; 129.451; 129.592; 129.664; 130.151; 130.943; 152.336; 165.684 (arom. C); IR (KBr) cm^{-1}: 3100–3000 (*v* CH-arom.); 3000–2800 (*v* CH_2); 1624 (*v* C=N); 1556 (vibrations of heteroarom.ring); 1446 (δ CH, crown); 1348 (*v* C-N, crown); 1110, 1103, 1077 (*v* C-O-C, crown); 822, 795 (γ CH-arom).

2-Dimethylaminobenz[c,d]indol (9). Initially 6 mmol of dimethylamine as a 30% aqueous solution were added to a suspension of 2 mmol of **1** in methanol. The reaction mixture was stirred at 20–22°C for 5 h. The orange solid was filtered, washed with water, dried and purified by column chromatography with benzene and recrystallized from benzene. The yield was 47% m.p. 128–129°C. Found: C, 79.50; H, 6.19; N, 14.40. Calc. for $C_{13}H_{12}N_2$: C, 79.55; H, 6.16; N, 14.28. MS: the base peak [M+H] was observed at 197.1081 (calculated for $C_{13}H_{12}N_2Na$: 197.253); ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.5 [(-N(CH_3)$_2$, s, 6H]; 7.28–7.31 (CH-8, d, J = 6.59 Hz, 1H); 7.39–7.48 (CH-7.6, t+d, 2H); 7.54–7.59 (CH-4, t, J = 7.5 Hz, 1H); 7.81–7.84 (CH-5, d, J = 7.2 Hz, 1H); 7.88–7.91 (CH-3, d, J = 8.05 Hz, 1H). ^{13}C-NMR ($CDCl_3$) δ (ppm) 40.3828 (-CH_2-N); 113.995; 119.250; 125.386; 127.207; 129.487; 129.569; 129.783; 130.178; 131.250; 152.501; 166.463 (arom. C). IR (KBr) cm^{-1}: 3046, 3018, 2998, 2920 (*v* CH arom.); 2798 (Fermiresonance band of -N(CH_3); 1572 (*v* C=N arom.); 1455, 1385 (δ CH_2); 1340 (v C-N); 822, 775 (γ CH).

RESULTS AND DISCUSSION

Various crown ether analogues containing the heteroatoms oxygen and nitrogen efficiently complex a great variety of metal ions and a number of fluoroionophores functionalized by such moieties are well known [28–30]. The synthesis, properties and structure of such ionophores based on diazacrown ethers has also been previously described [31–33]. Some of these probes were obtained under high dilution conditions [31] or by nucleophilic substitution reaction under high pressure [33, 34].

The electron-rich heterocyclic system benz[c,d]indole was chosen as a fluorophore because besides the actual receptor, the lone electron pair of its nitrogen atom could act as an additional complexation site [34]. Such systems are known to undergo more pronounced spectroscopic changes upon cation coordination and show higher complexation constants compared to molecules where the chromophore does not interact with the cation bound. Additionally, having in mind interferences from fluorescent matrices or other

fluorescent compounds in the sample media, we aimed at the design of ionophores absorbing and emitting in the visible region of the spectrum without extending the size of the chromophore at large. Due to its own deep absorption and relatively small heterocyclic nucleus, the benz[c,d]indole moiety matches these requirements well [35].

The synthesis of the new functionalized azacrown ethers was performed using 2-methylthiabenz[c,d]indolium iodide **1** [36].

As follows from Fig. 1, the general procedure for the synthesis of these functionalized azacrown ethers involved heating the mixture of salt **1** and the corresponding aza- and diazacrown ethers **2a–2d** in aprotic polar solvents (dimethylsulfoxide, dimethylacetamide) at 110°C for 2 h, in some cases in the presence of triethylamine. The reaction products were purified by column chromatography. The model compound 2-dimethylaminoben[c,d]indole was synthesized from **1** and dimethylamine.

The structures of all new substances were confirmed by UV-, IR-, NMR- and mass spectroscopy and their purity was checked by reversed-phase HPLC with UV detection. As expected, compounds **3–9** show absorption maxima in the visible region of the spectrum at 411–419 nm in methanolic

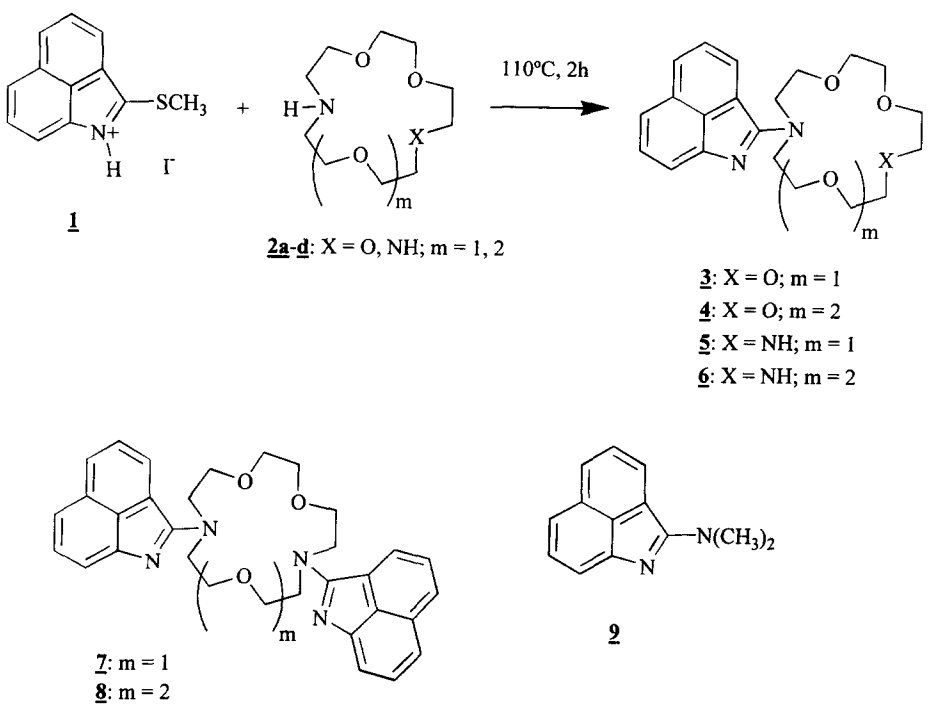

Fig. 1. Synthetic scheme for the preparation of the benz[c,d]indole containing fluorescence probes.

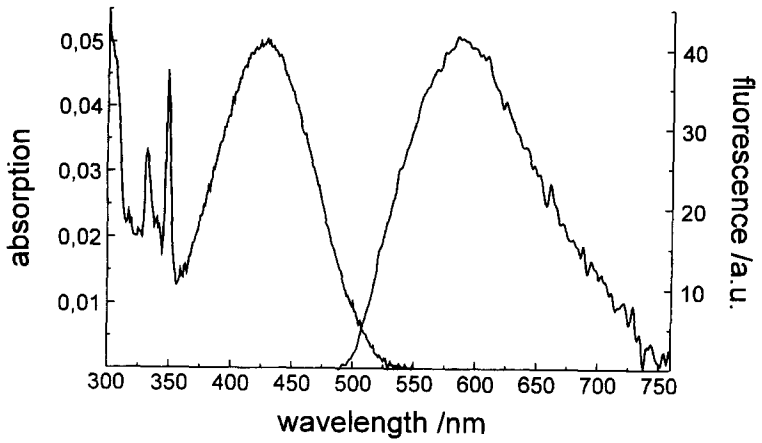

Fig. 2. Absorption and emission spectrum of **5** in acetonitrile: excitation at 427 nm.

solution and 427 nm in acetonitrile, respectively. The chemical structures of the molecules investigated are shown in Fig. 1.

The spetroscopic behaviour of compounds **3**, (BI-A15C5), **5** (BI-$A_2$15C5), **7** (BisBI-$A_2$15C5), and **9** (BI-DMA) and some of their complexes with heavy and transition metal ions were investigated in acetonitrile. These four compounds show nearly identical absorption and emission bands peaking at 427 nm and *ca* 590 nm, respectively (see Fig. 2). The fluorescence quantum yields of all compounds are comparatively low and their fluorescence decays monoexponetially with a lifetime of 0.5 to 0.6 ns. The spectral data of the four compounds are summarized in Table 1; the radiative and non-radiative rate constants were calculated via the photophysical equations $k_r = f_f/t_f$ and $k_{nr} = (1-f_f)/t_f$.

On addition of various heavy and transition metal ions a hypsochromic shift in both absorption and emission occurs, the magnitude of the shift depending on the probe and on the cation bound. Interestingly, complexation not only leads to fluorescence enhancement and an increase in fluorescence lifetime, but also increases the molar extinction coefficient of the

TABLE 1
Spectroscopic Data of **3**, **5**, **7**, and **9** in Acetonitrile: For Calculation of k_r and k_{nr} See Text

	$\lambda_{max}(abs)$/ nm	ε_λ/ $l\,mol^{-1}\,cm^{-1}$	$\lambda_{max}(em)$/ nm	$\phi_f \times 10^{-3}$	τ_f/ns	$\Delta\upsilon_{a-e}$[a]/ cm^{-1}	k_r/ $10^{-11}s^{-1}$	k_{nr}/ $10^{-11}s^{-1}$
3	427	4848	590	4.0	0.55	6470	0.73	181
5	427	4919	589	4.6	0.52	6440	0.88	191
7	427	9015	590	5.1	0.51	6470	1.00	195
9	427	4198	589	6.6	0.62	6440	1.06	160

[a] $\Delta\upsilon_{a-e}$ = Stokes shift.

TABLE 2
Spectroscopic Data of Pb^{2+}-Complexes of **3**, **5**, **7**, and **9** in Acetonitrile

	λ_{max} (abs)/ nm	ε_λ/ 1 mol^{-1} cm^{-1}	λ_{max} (em)/ nm	ϕ_f	τ_f/ns[a]	$\Delta\upsilon_{a-e}$[b]/ cm^{-1}	$\Delta\upsilon_{c-p}$ (abs)[b]/ cm^{-1}	$\Delta\upsilon_{c-p}$ (em)[b] /cm^{-1}	CHEF
3⊂Pb^{2+}	385	8464	505	0.015	0.87	6170	2555	2766	3.9
5⊂Pb^{2+}	386	8326	505	0.136	1.27, 3.66	6100	2487	2766	29.4
7⊂Pb^{2+}	385	14681	517	0.151	4.29, 11.27	6630	2555	2306	29.5
9⊂Pb2+	379	8419	501	0.129	1.90	6420	2966	2953	19.4

[a]The relative amplitude of τ_1 and τ_2 depend on the metal ion concentration, the short lifetime increasing with increasing metal ion concentration.
[b]$\Delta\upsilon_{a-e}$ = Stokes shift; $\Delta\upsilon_{c-p}$ = shift between band of the complex and the free probe.

system. The spectral data of some of the complexes investigated are shown in Tables 2 and 3. Figures 3(a) and (b) show UV/Vis-spectrophotometric titrations of **5** (Fig. 3(a)) and **9** (Fig. 3(b)) with Pb^{2+}, Fig. 4 the corresponding fluorometric titration of 5 with Pb^{2+}.

The dependence of the absorption and emission maximum of a given fluoroionophore on the nature of the heavy metal ion could be derived from Table 3 and is illustrated by the absorption and emission spectra of **5** in the presence of a 10-fold excess of Pb^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Cu^{2+}, and Ni^{2+} in Figs 5 and 6. For comparison, the spectra of the protonated ionophore are included.

TABLE 3
Spectroscopic Data of M^{2+}-Complexes of 5 in Acetonitrile

	λ_{max} (abs)/ nm	ε_λ/ 1 mol^{-1} cm^{-1}	λ_{max} (em)/ nm	ϕ_f	τ_f/ns[a]	$\Delta\upsilon_{a-e}$/ cm^{-1}	$\Delta\upsilon_{c-p}$ (abs)[b]/ cm^{-1}	$\Delta\upsilon_{c-p}$ (em)/ cm^{-1}	CHEF
5⊂Pb^{2+}	386	8326	505	0.136	1.28, 3.49	6100	2490	2850	29.4
5⊂Zn^{2+}	383	8478	505	0.138	1.76, 4.15	6310	2690	2766	30.0
5⊂Cd^{2+}	403	5860	523	0.058	1.37, 3.90	5690	1390	2170	12.5
5⊂Hg^{2+}	385	8478	507	0.128	1.88, 4.70	6250	2550	2770	27.8
5⊂Cu^{2+}	378[c]	5730	506	0.130	2.71, 7.11	6550	3030	2810	28.3
5⊂Ni^{2+}	410	4990	505	0.017	3.52	4590	970	2850	3.6

[a]The relative amplitude τ_1 and τ_2 depend on the metal ion concentration, the short lifetime increasing with increasing metal ion concentration.
[b]$\Delta\upsilon_{a-e}$ = Stokes shift; $\Delta\upsilon_{c-p}$ = shift between band of the complex and the free probe.
[c]Temporal dependence of the absorption band: shift from 390 nm (1 min after addition) to 380 nm (18 h after addition) and additional blue band for Cu^{2+} at 610 nm (ε_λ = 5643 1 mol^{-1}cm^{-1}).

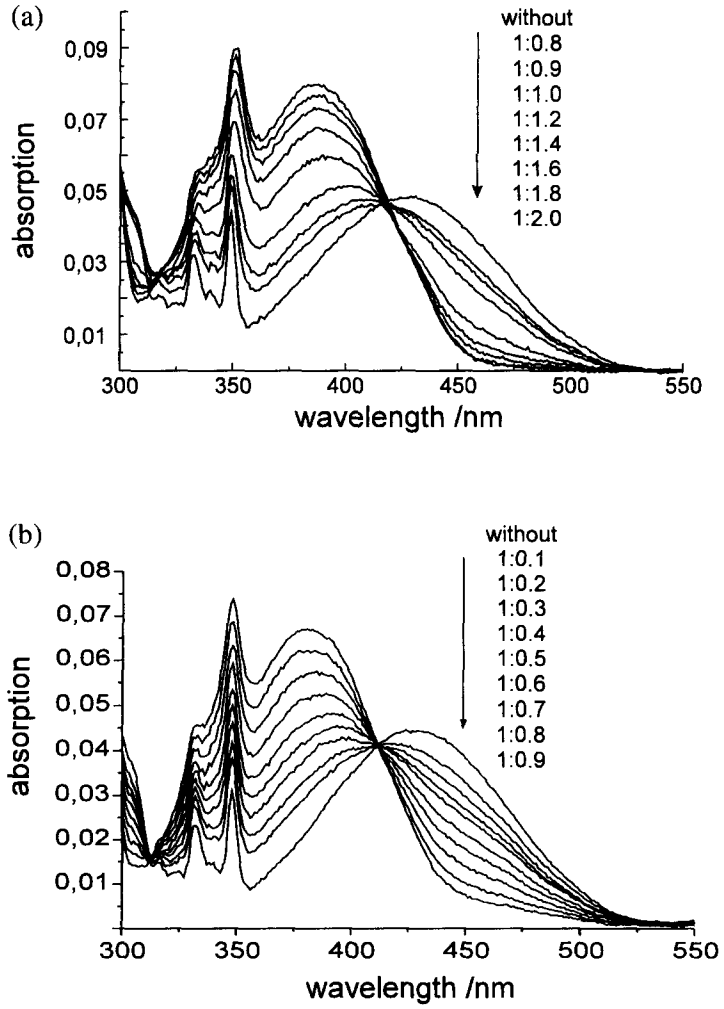

Fig. 3. (a) UV/Vis-spectrophotometric titration of **5** with Pb^{2+} in acetonitrile: $c_5 = 1 \times 10^{-5}$ mol l^{-1}, ratio **5**:Pb^{2+} indicated. (b) UV/Vis-spectrophotometric titration of **9** with Pb^{2+} in acetonitrile: $c_5 = 1 \times 10^{-5}$ mol l^{-1}, ratio **9**:Pb^{2+} indicated.

In the case of all probes the complexes with Pb^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+} reached the absorption and emission maxima of the protic salt, 385 nm/505 nm for **3**, **5**, and **7**, 379 nm/501 nm for **9**, the only exception being the complex of 5 and Cd^{2+}, which shows a less pronounced hypsochromic shift (see Table 3 and Fig. 4). For all probes, Ni^{2+} shows a less pronounced hypsochromic shift only in absorption. Complexation to Cu^{2+} is accompanied by a slightly more pronounced hypsochromic shift in absorption for **5** and **7** but both shifts are less pronounced (i.e. for **3**: Dn_{c-p} (abs) = 2290 cm^{-1}, Dn_{c-p} (em) = 2220 cm^{-1}) for **3** and **9** and additionally, the appearance

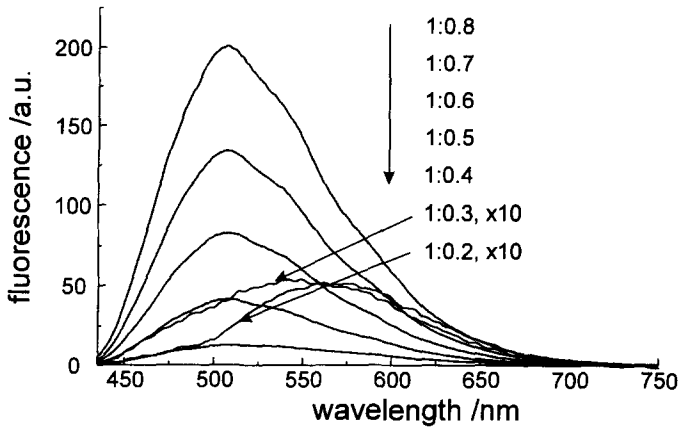

Fig. 4. Fluorometric titration of **5** with Pb^{2+} in acetonitrile: $C_5 = 1 \times 10^{-5}$ mol l^{-1}, ratio **5**:Pb^{2+} indicated.

of a second, bathochromically shifted intense absorption band at 610 nm (e_1, *ca* 56001 mol^{-1} cm^{-1}) is observed. This band decreases with time and is absent after *ca* 18 h.

CHEF factors are only comparatively small for **3** (*ca* 1–4) and are in the range of 10–30 for most metal ions including the commonly known fluorescence quenchers Cu^{2+} and Hg^{2+} in the case of **5**, **7**, and **9**. For all complexes, the shifts between the absorption and emission band of the free probe and the complex are similar. Fluorescence decays of the complexes of **3** and **9** are monoexponentially (exception: Cu^{2+}) a discrimination between different complexes being only possible in the case of **9**. The fluorescence of

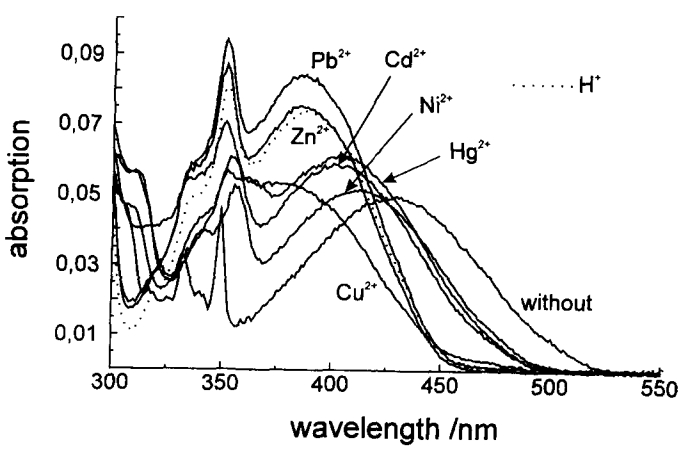

Fig. 5. Dependence of the absorption spectrum of **5** on the nature of the cation: $c_5 = 1 \times 10^{-5}$ mol l^{-1}, 10-fold excess of M^{2+}; spectrum of the protonated probe included (dotted line).

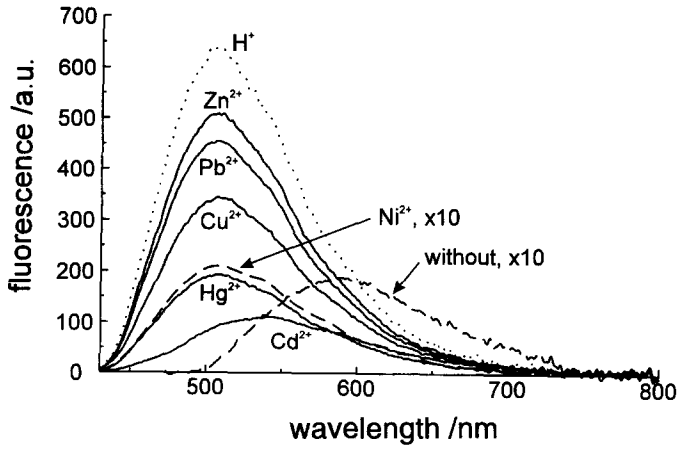

Fig. 6. Dependence of the emission spectrum of **5** on the nature of the cation: $c_5 = 1 \times 10^{-5}$ mol l^{-1}, 10-fold excess of M^{2+}; excitation at the isosbestic points, spectra corrected for optical density at the excitation wavelength, spectrum of the protonated probe included (dotted line).

the complexes of **7** always decays biexponentially, for **5** mono-and biexponential decays are observed depending on the nature and concentration of the cation (Fig. 7).

The stoichiometry of the complexes is 2:1 for **3** and **9**, resulting in well-defined isosbestic points for spectrophotometric titrations, i.e. at 420 nm (**3**) and 415 nm (**9**) for the Pb^{2+} complexes (Fig. 3b). A characteristic value of 0.67 is obtained when plotting the absorption of different mixtures of equimolecular solutions of probe and cation versus. the mole fraction of the

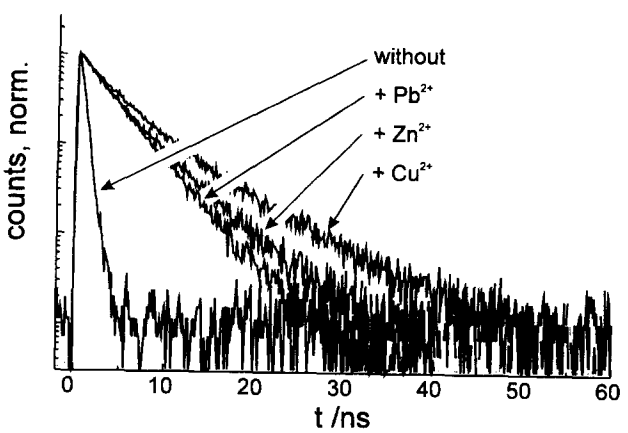

Fig. 7. Fluorescence decay curves of **5** and its complexes with Pb^{2+}, Zn^{2+}, and Cu^{2+}. Excitation at 415 nm with 100μW, emission at 500 nm, decays normalized.

probe (Fig. 8) [37]. A different behaviour is shown by **5** upon complexation to the cations investigated. In the case of Zn^{2+} and Cd^{2+} isosbestic points at 416 nm and 436 nm are observed, whereas for Pb^{2+} and especially for Hg^{2+} no such distinct points occur. Following the method of continuous variation for the determination of the composition of the Pb^{2+} complex of **5** a stoichiometry of **5**: $Pb^{2+} = 1{:}2$ is found, independent of the chosen wavelength [38]. This different complexation behaviour implies a larger dynamic range for chelation-induced spectroscopic changes. Whereas full complexation is achieved for a ratio of 1:1 to 3:1 of probe and ion for **3**, **7** and **9**, this range covers two orders of magnitude for **5** (Fig. 9). Complexation kinetics are within the seconds time domain for **3**, **5** (except for Cd^{2+}: all measurements were performed after incubating the solutions at 55°C for 10 min), and **9** with all ions investigated, but slower kinetics are observed for **7**. Stability constants of some of the complexes investigated are presented in Table 4. The above mentioned results of the time-resolved fluorescence measurements are in coincidence with the complex formation kinetics. Global analysis of a set of fluorescence decays recorded at various emission wavelengths always yielded one or two species decaying with $\tau_f >> 0.6$ns and emitting at shorter wavelengths than the free ionophore, which could be ascribed to the formed complexes. No rise times have been found in any case, therefore a reaction in the excited state on the >ps time scale could be excluded.

The differences in complexation-induced spectroscopical changes could be understood on the basis of the different chelation sites offered by the inves-

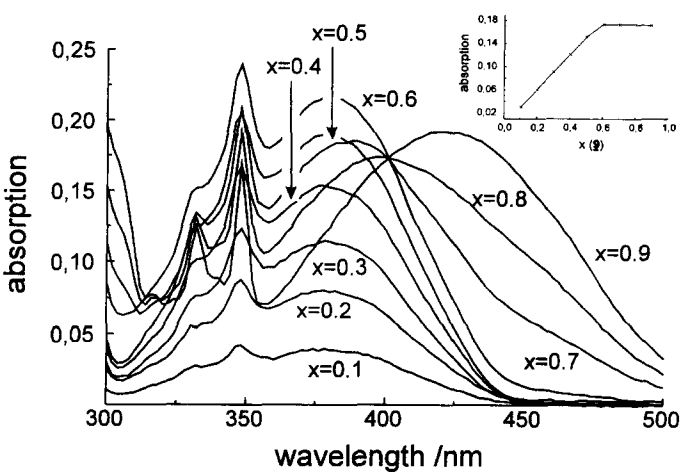

Fig. 8. Plot of the absorption spectrum of 9-Pb^{2+}-mixtures as a function of the mole fraction of **9**. x(**9**) M + y(Pb^{2+}) M = 1, $c_0 = 5 \times 10^{-5}$ mol l^{-1}. Insert: Plot of absorption at the isosbestic point (401 nm) versus. the mole fraction of **9**.

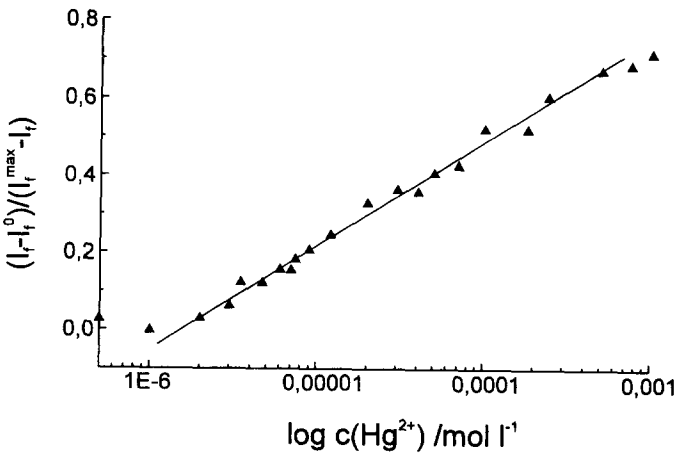

Fig. 9. Dependence of fluorescence intensity on Hg^{2+}-concentration for **5** in acetonitrile: $c_5 = 1 \times 10^{-5}$ mol l^{-1}

tigated compounds. In the case of **9** only chelation to the benz[c,d]indole nitrogen and/or to the dimethylamino nitrogen is possible. Since the A15C5 moiety does not show a high affinity towards heavy metal ions, and therefore does not compete very efficiently with the nitrogen donor atoms, **3** shows a comparable complexation behaviour. For **5** and **7**, the $A_2$15C5 heteromacrocycle, with considerably high complexation constants for the metal ions investigated, is the main chelation site [39]. Furthermore, in these probes the 2-benz[c,d]indolyl-substituent can provide an additional donor atom for ligation. Showing also large complexation-induced shifts in the excited state ($\Delta\nu_{c-p}$ (em) in Tables 2 and 3) the chelates formed should show high complexation constants in the excited state as well.

Recently, Tsukube *et al.* reported on the investigation of ^{13}C-NMR spectra for diazacrown ether complexes functionalized by 2-thiazolyl substituent.[33] They observed changes in chemical shifts for the C-atoms of the >N-CH fragment in the macrocycle. Based on this fact they concluded that the nitrogen atom of the macrocycle takes part in the complexation. Our spectral data confirm this assumption. The large CHEF factors of *ca* 30 for most of the cations investigated suggest that, upon complexation, the electron transfer in the excited state from the exocyclic nitrogen atom to the naphthalene heteroaromatic system of the benz[c,d]indole is inhibited, and a 'switching on' of fluorescence is achieved [5]. The 2-benz[c,d]indole fragment is rigidly fixed relative to the macrocyclic plane, and therefore the energy barrier for the rotation around the [C(2)-N macrocyclic]-bond is increased, leading to a reduced efficiency in non-radiative transitions. In the case of **9** both nitrogen atoms—exocyclic as well as the indole nitrogen of the benz[c,d]indole resi-

due—take part in the complexation, the basic indole nitrogen playing the major role. Due to steric hindrances of the crown moiety, in the case of **3** additional chelation to the crown nitrogen should be less favoured. The exceptional behaviour of complexes of **3** in the excited state, a 10-fold weaker CHEF and a comparatively small increase in fluorescence quantum yield and lifetime, results in both a much smaller increase in k_r, and decrease in k_{nr} than observed for the complexes of the other ionophores. As mentioned above, upon complexation to the same cations, nearly similar shifts are observed for **3**, **5**, **7**, and **9**. This is evidence in favor of the assumption that the indole nitrogen participates in chelation in all cases. Investigations involving alkali and alkaline-earth metal ions support this assumption as well. For alkali metal ions, even for a 1000-fold excess only a weak hypsochromically shifted shoulder is visible in the emission spectra of **3** and **9**, no effect being detectable for **5** and **7**. Alkaline-earth metal ions show the same effects as heavy metal ions only at a 10- to 100-fold higher concentration depending on the fluoroionophore. Furthermore, protonation of the ionophores occurring at both nitrogens leads to the same spectral shifts and enhancements in both absorption and fluorescence.

CONCLUSIONS

Covalently linking the fluorophore benz[c,d]indole to one or two nitrogen atoms of various macrocyclic receptors yields a series of fluoroionophores whose absorption and fluorescence properties are significantly changed upon cation complexation of the receptor. Besides hypsochromic shifts in both absorption and fluorescence, an increase in molar extinction coefficient, fluorescence quantum yield and lifetime is observed. Considering complexation kinetics, dynamic range for both ion concentration and CHEF factors, ionophore **5** possesses the best features for applications as a fluorescent label in chromatography. Furthermore, the differences in fluorescence decay times for the complexes of **5** provide a very useful tool for the determination of analytes in IEC via a second, chromatographically independent parameter. In addition, all systems presented show large CHEF effects on complexation to the well-known fluorescence quenchers such as Cu^{2+}, Hg^{2+} and Ni^{2+}.

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